

## Biofilm system 3

# Differentiation and colistin tolerance in *Pseudomonas aeruginosa* biofilms.

### Introduction

One of the most important aspects of microbial biofilm biology is that the bacteria in these sessile communities display a remarkable increased tolerance to antimicrobial attack. Because of their innate resistance to host immune systems, antibiotics and other biocides, biofilms in medical and industrial settings are difficult, if not impossible, to eradicate. Biofilm formation therefore leads to various persistent and sometimes lethal infections in humans and animals, and to a variety of problems in industry where solid-water interfaces occur. Detailed knowledge of the developmental process from single cells scattered on a surface to the development of antimicrobial-tolerant biofilms is essential in order to create strategies to control biofilm development.

Structural biofilm development by *P. aeruginosa* appears to be conditional. For example, in flow-chambers irrigated with citrate minimal medium *P. aeruginosa* forms a flat biofilm, while in flow-chambers irrigated with glucose minimal medium it forms a heterogeneous biofilm with mushroom-shaped multicellular structures.

The formation of the flat *P. aeruginosa* biofilm in flow-chambers irrigated with citrate minimal medium was shown to occur via initial formation of microcolonies by clonal growth of sessile bacteria at the substratum, followed by expansive migration of the bacteria out on the substratum, resulting in the formation of a very dynamic flat biofilm. Since biofilm formation by a *P. aeruginosa pilA* mutant (which is deficient in biogenesis of type IV pili) occurred without the expansive phase, and resulted in discrete protruding microcolonies, it was suggested that the expansive migration of the bacteria out on the substratum was type IV pili-driven.

The formation of the mushroom-shaped structures in the glucose-grown *P. aeruginosa* biofilm was shown to occur in a sequential process. This involves a non-motile bacterial sub-population which form the initial microcolonies by growth in certain foci of the biofilm, and a migrating bacterial sub-population which initially form a monolayer on the substratum. Subsequently they form the mushroom caps by climbing the initial microcolonies (which then become mushroom stalks) and aggregating on their upper surface via a process which is dependent on type IV pili. Development of the heterogeneous *P. aeruginosa* biofilm therefore evidently involves differentiation of the bacteria into at least two subpopulations (a motile and a non-motile).

## **Aims**

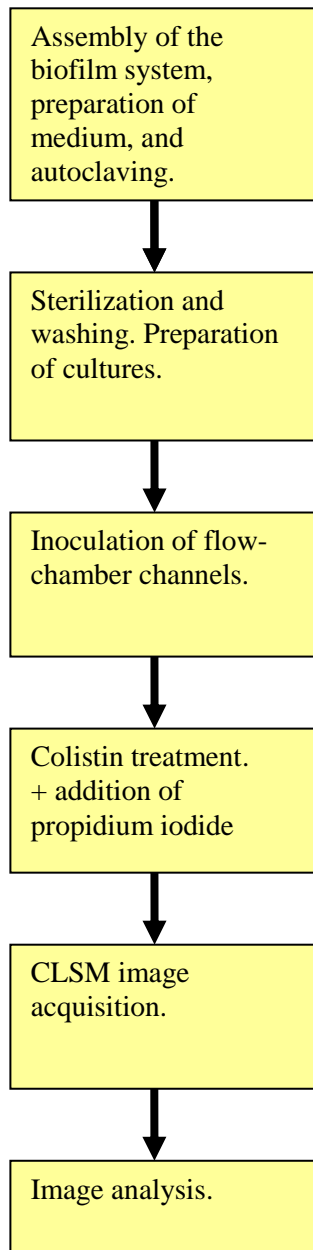
In the present exercise the motile and non-motile subpopulations in glucose-grown *P. aeruginosa* biofilms, forming cap and stalk of the biofilm mushrooms, are investigated with respect to tolerance to the antibiotic colistin. You are going to take images of the spatial distribution of *P. aeruginosa* wild type and non-motile PilA mutant in the mixed biofilm and of colistin treated and untreated *P. aeruginosa* biofilms. Afterwards the cells will be harvested from the channels and prepared for plating and flow cytometry.

## **References:**

Involvement of bacterial migration in the development of complex multicellular structures in *Pseudomonas aeruginosa* biofilms. Klausen M, Aaes-Jorgensen A, Molin S, Tolker-Nielsen T.

*Mol Microbiol.* 2003 Oct;50(1):61-8

Development of tolerance to anti-microbial agents in the motile subpopulation of *Pseudomonas aeruginosa* flow-cell biofilms. Haagenen JA, Klausen M, Ernst RK, Miller SI, Folkesson A, Tolker-Nielsen T, Molin S. *J Bacteriol.* 2007 Jan;189(1):28-37



## **Experimental procedures**

### **Day 0. Assembly of the biofilm system, preparation of medium, and autoclaving.**

See the protocol for biofilm work.

### **Day 0. Sterilization and washing of the biofilm system equilibrate tubing with media over night.**

See the protocol for biofilm work.

### **Day 0. Preparation of cultures.**

Each team will have one flow cell available (3 channels).

- **One channel with *P. aeruginosa*, Gfp tagged (as control),**
- **One channel with *P. aeruginosa*, Gfp tagged (treatment with colistin) and**
- **One channel with a mixture of *P. aeruginosa* (Yfp tagged) and *P. aeruginosa* pilA (Cfp tagged)**

The strains should be inoculated in 10mL LB and incubated O.N. at 30°C. These cultures will be used to inoculate biofilms on day 4.

### **Day 1. Inoculation of flow-chamber channels.**

See the protocol for biofilm work.

The O.N. cultures should be diluted 1000 times in 0.9% NaCl before inoculation.

### **Day 4. Colistin treatment.**

The 3 day-old PAO1 biofilm tagged with Gfp should be treated with the antibiotic colistin for 20 h (remember that one channel is used as control and should not receive antibiotic treatment). This is done by changing the medium irrigating the flow channels of interest to medium containing colistin in a final concentration of 25 µg/ml. Addition of 10 µl Propidium iodide to 1 L media (PI, 20mM/ml in DMSO) as Live/Dead indicator. *Note: Exchange also the medium in the bubble traps.*

Note that PI is light sensitive (-> Cover biofilm system with aluminium foil).

### **Day 5. CLSM image acquisition.**

All microscopic observations are performed by the use of a Zeiss LSM510 Confocal Laser Scanning Microscope (CLSM) equipped with lasers, filter sets, and detectors for simultaneous monitoring of Gfp (excitation 488 nm, emission 517 nm) and red-fluorescence emitted from the PI (excitation 543 nm, emission 565 nm); as well as Cfp (excitation 458 nm, emission 480-520nm) and

Yfp (excitation 514 nm, emission 535-590 nm). The images are acquired with a 40x/1.3 Plan-Neofluar oil immersion objective.

**Image analysis.**

Prepare images of the different biofilms using Imaris software.